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A New Era of Antifungal Therapy

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ABSTRACT

Invasive fungal infections pose major management problems for clinicians caring for hematopoietic cell transplant patients. Two major fungal genera, *Candida* and *Aspergillus*, account for most fungal infections. Rates of systemic *Candida* infection range from 15% to 25%, mostly in the pre-engraftment period. Prophylaxis by fluconazole has dramatically reduced the frequency of early *Candida* infections. Caspofungin has recently been shown to offer an excellent alternative to amphotericin B (with less toxicity) or fluconazole (with a broader spectrum) for therapy of systemic *Candida* infections. *Aspergillus* infections occur in 15% to 20% of allogeneic hematopoietic cell transplant patients, most frequently in the post-engraftment period; they are associated with a severe diminution of cell-mediated immune responses by graft-versus-host disease and prolonged corticosteroid use. Voriconazole, a recently introduced broad-spectrum azole, has excellent activity against *Aspergillus* and is generally well tolerated. Voriconazole currently offers the best prospect for success and tolerance as a first-line treatment for aspergillosis. Second-line therapies include lipid formulations of amphotericin B, caspofungin, or intravenous itraconazole. Unfortunately, early initiation of therapy for aspergillosis is frequently not possible because of inaccurate diagnostics. One new diagnostic, the galactomannan assay, has recently been approved, and others are in development; these offer promise for earlier diagnosis without the need for invasive procedures. It is hoped that these new therapies and new diagnostics will usher in a new era of antifungal therapy.

KEY WORDS

Antifungal therapy • *Aspergillus* • *Candida*

THE PROBLEM

The 2 most difficult challenges facing the clinician caring for allogeneic hematopoietic cell transplant (HCT) recipients are graft-versus-host disease (GVHD) and infectious complications. Once cytomegalovirus (CMV) was the chief infectious threat, but today, invasive fungal infections (IFI) are the major causes of infectious morbidity and mortality after allogeneic HCT. The change is due not only to a diminution in serious CMV disease from the introduction of new drugs and adoption of new prophylactic or preemptive strategies, but also to an increase in IFI rates, changes in fungal epidemiology, and lack of progress in fungal therapeutic approaches.

A double peak in the occurrence of IFIs was noted many years ago. The first peak occurs during the first month after HCT (the pre-engraftment interval). *Candida*, a yeast, is part of the endogenous flora of patients and historically has been the most common fungal pathogen during the pre-engraftment period. Systemic invasion by colonizing *Candida* organisms takes place with bacterial suppression by antibiotics (permitting fungal overgrowth), mucosal injury by intensive conditioning regimens (allowing easier entry), and loss of second-line host defenses (phagocytosis compromised by neutropenia, and cell-mediated immunity suppressed by the immunosuppressive regimen and GVHD). *Aspergillus*, a mold, is an exogenously acquired pathogen that usually gains entry by inhalation into nasal passages and the respiratory tract. *Aspergillus* represents a distant second pathogen during the pre-engraftment period.

The second peak occurs in the postengraftment period, chiefly during the second and third months.

GVHD and the use of corticosteroids are the chief risk factors. *Aspergillus* is the predominant pathogen in this second peak; *Candida* and other mold pathogens account for a minority of the other IFIs.

Other risk factors for IFIs have been identified. Use of more intensive conditioning regimens, prior aspergillosis, CMV infection, use of T-cell depletion of the stem cell graft, use of cord blood as the source of stem cells, and HLA mismatching of donor and recipient are among the risk factors noted in various series.

In recent years, a number of changes have occurred in rates of infection, types of pathogens, and time course. *Candida* infections have dramatically decreased with the adoption of fluconazole prophylaxis during the pre-engraftment period. Hematopoietic growth factors and the use of peripheral blood to optimize the $CD34^+$ cell content of the stem cell graft have shortened the time to engraftment. Reduced-intensity nonmyeloablative conditioning regimens have reduced the duration of neutropenia and the degree of damage to the mucosa of the gastrointestinal tract. These changes in transplantation practice have all combined to greatly reduce the risk for IFI (as well as bacterial infections) during the pre-engraftment period. In contrast, several changes in transplantation practice have combined to increase the risk for IFIs in the postengraftment period. The increasing use of alternate allogeneic donors, including matched unrelated donors, mismatched family donors, and cord blood, and the reliance on more potent immunosuppressive regimens to suppress GVHD have increased the risk for IFI after engraftment. The higher rates of chronic GVHD after peripheral blood allografts have been accompanied by more IFIs, along with other infectious complications. Today, more and more IFIs are occurring after day 100, extending the duration of vulnerability.

For autologous HCT recipients, certain events before transplantation place the patient at risk. Prior IFI is associated with an almost guaranteed exacerbation during subsequent HCT. Potent purine analogs also increase the susceptibility for IFI by producing a profound, long-lasting deficiency of cell-mediated immunity. Events during the transplantation procedure, including $CD34^+$ cell selection of the stem cell graft and use of steroids during the peritransplantation period, increase the susceptibility of autologous HCT recipients for IFI in the postengraftment period.

The effectiveness of fluconazole prophylaxis in reduction of *Candida* infections has shaped the fungal therapy map for a decade. Although concerns were raised nearly a decade ago that emergence of fluconazole resistance would mitigate the effectiveness of *Candida* prophylaxis, that fear has fortunately largely remained unrealized. Scattered reports of several outbreaks of *Candida* infections have been noted in HCT patients receiving fluconazole because of resistant *Candida* species (*Candida krusei* and *C. glabrata*) and also because of fluconazole-sensitive species (*C. parapsilosis*), yet these have been largely isolated. Shifts to non-*albicans Candida* species have occurred, but the net effect of fluconazole has been an enormous reduction in the more prevalent *C. albicans* and *C. tropicalis* species; this net decrease to date has overshadowed the smaller increase in the non-*albicans* species.

Although *Candida* infections have decreased, *Aspergillus* infections have relentlessly increased in frequency. *Aspergillus* species once infected only 4% to 5% of allogeneic HCT recipients, but today rates of 12% to 15% are regularly being reported, and even higher rates have been observed in some centers and in some subgroups of high-risk patients. Although it was hoped that nonmyeloablative allogeneic transplants would be associated with fewer infections, a decrease in IFIs has not been realized in several reported series. In part, the high rate of IFI after nonmyeloablative allogeneic transplantations is related to the more aggressive tapering of immunosuppressive therapy in many such regimens that is designed to maximize the potential for graft-versus-tumor effects. Many of the early series also were composed of high-risk patients with multiple failed attempts to control the underlying disease or a higher tumor burden at the time of transplantation or of frail patients with multiple comorbidities. With time, as regimens are standardized and lower-risk groups undergo transplantation, this may change.

Infections from other mold pathogens have also increased, including *Zygomycetes* and *Fusarium* species, but still these are relatively infrequent. Infections due to *Scedosporium* species have remained infrequent.

The time of onset has gradually been pushed to later times. The apparent increase in late-onset IFI is in part attributable to the abrogation of the initial pre-engraftment peak from *Candida*, but it is also attributable to the greater occurrence of chronic GVHD with peripheral blood grafts and to an increase in older patients receiving transplants. This later onset frequently takes place after the patient has left the transplant center, which poses additional challenges for close monitoring and vigilance for longer periods and often at great distances from the transplant center.

THE DRUGS

There are 4 classes of licensed drugs for therapy of IFIs. These include polyenes (the various formulations of amphotericin B), nucleoside analogs (flucytosine), azoles (fluconazole, itraconazole, and voriconazole), and echinocandins (caspofungin). Several of these drugs have established roles, and others have shown promise in HCT patients.

Table 1. *Mechanisms of Action of Different Antifungal Agents*

Class	Fungal Target	Action	Mechanism of Resistance
Polyene	Ergosterol	Binding to ergosterol	Altered or decreased amounts of ergosterol in cell membrane; defects in sterol biosynthetic pathways
Flucytosine	Nucleic acid synthesis	Inhibition of nucleic acid synthesis	Mutations in cytosine deaminase; decrease in uracil phosphoribosyl transferase activity
Azoles	Ergosterol	Inhibition of ergosterol biosynthesis	Mutations in ERG11, CDR1, CDR2, MDR1; overexpression of efflux pumps; overexpression/mutations of target enzyme
Echinocandins	β (1, 3) glucan synthetase	Inhibition of glucan biosynthesis	No data

Polyenes

Amphotericin B deoxycholate. Amphotericin B deoxycholate has the longest track record as an antifungal agent. Its mechanism of action is binding to sterols in the fungal cell membrane (Table 1). After it binds to ergosterol, leakage of intracellular univalent and divalent cations and subsequent cell death follow. This agent is preferentially more toxic to the fungal cell membrane than to the mammalian cell membrane because of its selectivity for ergosterol (in fungi) over cholesterol (in mammalian cells). Resistance to amphotericin B occurs infrequently but seems to take place by alteration in the ergosterol content in the fungal cell membrane [1,2].

Amphotericin B has the widest spectrum of activity against fungi, with activity against most human fungal pathogens, including most *Candida* and *Aspergillus* species. Amphotericin B was introduced 50 years ago; its efficacy was not tested in controlled trials, but for many years it was the only antifungal treatment option (see reviews [3,4]). Notwithstanding its wide spectrum of activity, there are several notable exceptions. Strains of *C. lusitaniae*, *C. guilliermondii*, *Trichosporon beigelli*, *A. terreus*, some strains of *A. flavus*, *Fusarium* species, *Pseudallescheria boydii*, and *Scedosporium* species are relatively resistant, and treatment outcomes are poor.

The pharmacokinetics of amphotericin B are poorly understood (Table 2). Much of the administered dose of amphotericin is deposited in tissues, especially fat, and a depot effect is notable with a slow release into the blood long after cessation of admin-

Table 2. *Pharmacologic Comparison of Most Commonly Used Agents*

Characteristic	Amphotericin B	Fluconazole	Itraconazole	Voriconazole	Caspofungin
Formulation	IV	PO, IV	PO, IV	PO, IV	IV
Yeast activity	Yes	Yes	Yes	Yes	Yes
Mold activity	Yes	No	Yes	Yes	Yes*
Half-life (h)	?	30	30-40 PO 35 IV	6	1-2†/9-11‡/40-50§
Loading dose	No	No	Yes	Yes	Yes
			200 mg PO TID × 3 d or 200 mg IV BID × 4 d	6 mg/kg IV Q12h × 2 doses or 400 mg PO Q12h × 2 doses	70 mg × 1 d
Usual maintenance dose	0.5-1 mg/kg	400 mg	200 mg	200 mg	50 mg
Frequency of dosing	Daily	Daily	Every 12 h	Every 12 h	Daily
Bioavailability of oral absorption	NA	>90%	55% (solution)	96%	NA
Route of elimination	Renal, fecal	Hepatic metabolism, renal excretion	Hepatic	Hepatic	Hepatic
CNS penetration	Yes#	Yes	No‖	Yes	No‖

NA indicates not applicable; IV, intravenous; PO, orally; CNS, central nervous system.

*Aspergillus only.

†Alpha half-life.

‡Beta half-life.

§Gamma half-life.

‖Inflamed meninges; poor penetration in uninflamed meninges.

#Clinical relevance not certain.

istration. Excretion occurs in urine and feces. Amphotericin B doses of approximately 0.5 to 0.75 mg/kg/d are generally sufficient to treat most *Candida* infections. Breakthrough *Aspergillus* infections have been noted in patients receiving amphotericin B doses of 0.5 mg/kg/d. Higher doses, in the range of 1 to 1.5 mg/kg/d, are necessary for treatment of *Aspergillus* and other mold infections.

Toxicities are frequent with amphotericin B. Infusional reactions include fever and rigors that occur in approximately half of all treated patients and, less frequently, hypotension, wheezing, hypoxia, and rash. Older age and rapid infusions are factors associated with a greater propensity for infusional reactions. Premedication with acetaminophen and diphenhydramine is frequently given to minimize the troubling reactions, but no controlled studies have demonstrated their effectiveness. Hydrocortisone and ibuprofen, in contrast, have been shown in controlled trials to reduce the reactions, although they are infrequently used because steroids may attenuate the effectiveness of antifungal therapy and ibuprofen is contraindicated in patients with thrombocytopenia. Fortunately, tachyphylaxis typically occurs, with or without premedication, and after 4 to 6 days the reactions tend to abate. Severe rigors can be treated with 25 to 50 mg of meperidine, although this agent should be avoided in patients with renal dysfunction because of accumulation of the neurotoxic metabolite (normeperidine), which may result in seizures.

Nephrotoxicity is a major limitation of the clinical usefulness of amphotericin B. The risk for nephrotoxicity varies in different series. Risk factors include the mean daily dose (≥35 mg), the duration of the treatment course, chronic renal disease, and the use of concomitant nephrotoxins such as cyclosporine and aminoglycosides. Slower infusions seem to be associated with reduced rates of nephrotoxicity [5-7]. Severe nephrotoxicity resulting in hemodialysis is associated with death [8,9] and results in higher utilization of health resources, prolonged hospital duration, and greater costs [8,10]. Even lower levels of nephrotoxicity, such as a doubling of the serum creatinine, are similarly associated with greater health-care resource utilization [11].

Saline loading has been shown to reduce the risk for nephrotoxicity [12]. Mannitol has not been proven to be useful [13]. Dopamine and sodium bicarbonate have been suggested to be protective against nephrotoxicity in preclinical models [14,15], but these have been evaluated in few clinical trials [16].

Other toxicities include anemia, electrolyte wasting by renal tubules (potassium, magnesium, and bicarbonate), hepatic dysfunction, seizures, and anorexia. One small study suggested that amiloride protected against amphotericin B–induced potassium wasting or at least reduced the daily potassium-replacement requirements [17].

The high rates of nephrotoxicity with amphotericin B in allogeneic HCT patients receiving calcineurin inhibitors have severely limited the ability of transplant clinicians to use amphotericin B in the therapy of IFIs. Thus, alternatives have been sought as high priorities for supportive-care measures.

Lipid formulations of amphotericin B. Lipid formulations of amphotericin B were developed to provide a less toxic formulation of amphotericin B. Three lipid formulations have been licensed in the United States: amphotericin B lipid complex (ABLC), amphotericin B in colloidal dispersion (ABCD), and liposomal amphotericin B (see reviews [4,18-23]). These agents all have the same antifungal spectrum of activity as amphotericin B deoxycholate.

The pharmacokinetic properties differ between the formulations, and interesting differences in tissue distribution of amphotericin B have been noted. Liposomal amphotericin B has been associated with substantially higher levels of amphotericin B in the brains of experimentally infected animals [24]. Autopsy analysis of amphotericin B tissue levels suggests substantially higher levels of amphotericin B in the lung after ABLC administration [18]. To date, the clinical significance of these observations has not been elucidated (see discussion [23]). Preclinical data suggest that higher concentrations of the lipid formulations compared with the deoxycholate formulation are needed to exert similar antifungal effects (see review [4]). Fortunately, the agents have substantially greater tolerability, permitting higher doses in clinical practice, and doses of 4 to 6 mg/kg/d were chosen for treatment of IFIs in most clinical trials.

All of the lipid formulations have been found in randomized trials to have substantially less nephrotoxicity than amphotericin B deoxycholate, even though substantially higher doses were used (4-6 mg/kg/d) [25-32] (Table 3). In 2 controlled trials comparing the toxicity of ABLC and liposomal amphotericin B when used as empirical therapy for neutropenic fever, liposomal amphotericin B was associated with less nephrotoxicity than ABLC [33,34]. Most of the differences seen between the 2 products were in mild degrees of nephrotoxicity, but there was also a smaller, but significant, difference in moderately severe nephrotoxicity. There were no differences in the rates of hemodialysis occasioned by severe nephrotoxicity, which was very infrequent with both products.

Infusional toxicities are less frequent with liposomal amphotericin B compared with amphotericin B deoxycholate [35] and with ABLC [34]. However, even with liposomal amphotericin B, there are infusional reactions, especially flank, chest, or back pain, which can occur in approximately 5% of patients [36]. Infusional reactions with ABCD are either similar to

Table 3. *Comparison of Lipid Amphotericin B Formulations with Amphotericin B Deoxycholate as First-Line Therapy of Invasive Fungal Infections*

Author	Pathogen	Agent	Response	Survival	Toxicity
Anaissie [28]	***Candida***	**ABLC**	**Same**	**Same**	**Less**
Bowden [31]	***Aspergillus***	**ABCD**	**Same**	**Same**	**Less**
Leenders [30]	**Mixed**	**L-amph**	**Same**	**Same**	**Less**
Hamill [27]	***Cryptococcus***	**L-amph**	**Same**	**Same**	**Less**
Leenders [29]	***Cryptococcus***	**L-amph**	**Same**	**Same**	**Less**
Johnson [26]	**Histoplasmosis**	**L-amph**	**Improved**	**Improved**	**Less**

ABLC indicates amphotericin B lipid complex; ABCD, amphotericin B colloidal dispension; L-amp, liposomal amphotericin.

or greater than those with amphotericin B deoxycholate [31,32].

Controlled trials of the amphotericin B lipid formulations with amphotericin B deoxycholate have not shown convincing superiority in response or survival rates in the first-line treatment of *Candida* [28], *Aspergillus* [31], or *Cryptococcus* [27,29], but liposomal amphotericin B was found to be superior to amphotericin B deoxycholate against histoplasmosis in human immunodeficiency virus–infected individuals [26] (Table 3). Despite no clear demonstration of superiority in efficacy, the advantages in safety and reductions in health-care resource utilization realized by the sparing of nephrotoxicity that offset much of the added cost of the drug, as well as the frank intolerance of amphotericin B deoxycholate, have led many HCT clinicians to abandon amphotericin B deoxycholate in favor of one of the lipid formulations [4]. Different clinicians have placed different weights on how much concern they place on infusional toxicities, how much incremental difference in nephrotoxicity they are willing to tolerate, and how much difference in price they are willing to bear in the choice of which lipid formulation to choose (see discussion [23]).

Dose schedules in controlled trials have varied considerably. Generally speaking, doses of 4 to 6 mg/kg/d have been used for the treatment of documented IFIs, whereas lower doses (1-3 mg/kg/d) have been studied in trials of empirical antifungal therapy for neutropenic fever. Only 1 trial has studied what dose schedule is optimal for the treatment of documented infection. Ellis et al. [37] evaluated liposomal amphotericin B in a randomized trial of 2 doses (1 and 4 mg/kg/d). Patients were required to have probable or proven invasive aspergillosis. No differences in either response rate or survival were seen, but it is important to note that the sample size was very small and the statistical power to detect a difference (if present) was inadequate to reject the null hypothesis; moreover, the response rate for proven infections in the 4 mg/kg/d group was higher than in the 1 mg/kg/d group (58% versus 37%). Many experts believe that this trial is inconclusive for recommending a lower-dose schedule for the therapy of invasive aspergillosis.

Lower-dose schedules were tested for empirical antifungal therapy for neutropenic fever [25,32,34,35]. These empirical therapy studies do not provide substantial evidence for efficacy differences between formulations; however, they do indicate that doses of 1 to 3 mg/kg/d are effective for empirical therapy, and they provide an excellent source of toxicity information. In one study, the toxicity of liposomal amphotericin B in doses of 3 and 5 mg/kg/d was not substantially different [34]. In another trial, liposomal amphotericin B given at 1 mg/kg/d was slightly less effective in defervescence than at a dose of 3 mg/kg/d [25]. Most evidence seems to suggest that the dose chosen should be governed by the indication for use rather than which lipid formulation is chosen: doses for documented infections range from 4 to 6 mg/kg/d, and doses for empirical antifungal therapy for suspected IFI during neutropenic fever range from 1 to 3 mg/kg/d.

Pyrimidine Analogs

Flucytosine is a pyrimidine analog that is transported by cytosine permease into susceptible fungi and then deaminated to the active form (5-fluorouracil), which subsequently interferes with fungal nucleic acid synthesis. *Candida* and *Cryptococcus* species are generally susceptible. Most molds (including *Aspergillus*) are resistant. As a single agent by itself, rapid development of resistance occurs through alteration in cytosine permease or altered metabolism [38]. Thus, its role has been relegated to an adjunctive role, and it has been most widely embraced as part of combination therapy of cryptococcal meningitis, along with amphotericin B. In vitro assays have suggested an additive effect to amphotericin B against *Candida* and *Aspergillus* species, but to date no clinical trial data have confirmed this.

Flucytosine is available only as an oral agent. Doses of 50 to 150 mg/kg/d (in divided doses every 6 hours) are generally used. The dose should be reduced in the presence of renal dysfunction. As noted, flucytosine is metabolized to fluorouracil, which is myelosuppressive and causes damage to the gastrointestinal mucosa. Thus, its use has been quite limited in the HCT setting. Moreover, because of variable bioavailability, levels should be monitored to avoid toxicity; blood concentrations should be maintained <100 μg/mL.

Azoles

The mechanism of action of the azole class of antifungals is inhibition of fungal cytochrome P450 14-α-sterol demethylase and of 24-methylene dihydrolanosterol demethylation (Table 1). These enzymes are key in the biosynthesis of ergosterol. With inhibition of the conversion of lanosterol to ergosterol, accumulation of lanosterol occurs, and there is a reduction of ergosterol in the fungal cell membrane, leading to inhibition of fungal growth. There are several azoles licensed for clinical use, and they differ by molecular structure according to specific side chains that lead to differences in pharmacologic properties, toxicity profiles, and spectra of activity. There are also several azoles in the investigational pipeline that are moving closer to commercial availability.

Fluconazole. Most *Candida* species are highly susceptible to fluconazole (defined as minimal inhibitory concentration ≤8 mg/L) [39]. However, several species are not reliably controlled by fluconazole. *C. krusei* is natively resistant to fluconazole. Isolates of *C. glabrata* are less susceptible to fluconazole and are generally classified as susceptible-dose dependent, meaning that higher concentrations are necessary for in vitro inhibition (minimal inhibitory concentration, 16 to 32 mg/L) rather than for susceptible isolates. Some *C. glabrata* strains are frankly resistant (≥64 mg/L). *C. dubliniensis* isolates are also resistant to fluconazole. Fortunately, *C. krusei* and *C. dubliniensis* infections are infrequent. However, *C. glabrata* infections account for 10% to 20% of all invasive *Candida* infections in various series, and these seem to be increasing over time. This increase seems coincident with the use of fluconazole, suggesting the pressures of selection of less susceptible fungal organisms. Reassuringly, bloodstream isolates of *C. albicans* have remained largely susceptible to fluconazole a decade after its introduction into clinical practice [40].

Resistance to fluconazole occurs through several mechanisms (see reviews [1,41]): alteration of the target enzyme (14-α-sterol-demethylase) by mutation or overexpression of ERG11 or upregulation of efflux transporters (encoded by CDR1, CDR2, and MDR1 genes). Emergence of resistance to *C. albicans* has been seen largely in patients with advanced acquired immunodeficiency syndrome with very low (and declining) $CD4^+$/T-lymphocyte counts, to whom prolonged administration of low doses (50-200 mg/d) of fluconazole was given for oropharyngeal candidiasis. This experience has contrasted with the experience in leukemia and HCT patients, in whom shorter courses of higher doses (400 mg/d) were given and in whom restoration of host defenses (neutrophil recovery, recovery of cell-mediated immune responses, or both) generally occurred. It seems likely that these different trajectories of host differences are important in understanding the reasons for these different experiences. Notwithstanding, as noted previously, several outbreaks of *Candida* bloodstream infections by fluconazole-resistant organisms have been reported in HCT patients receiving fluconazole prophylaxis [42-44]. Fortunately, these have been infrequent, and unpublished data exploring these outbreaks suggest that a common source may have been contributory. However, the resistance story in advanced human immunodeficiency virus infection should serve as a cautionary note for potential similar concerns that may pertain to patients with poor T-cell immune reconstitution after HCT.

All of the azoles are metabolized by the liver and are inhibitory to hepatic cytochrome P450 isoenzymes. Not surprisingly, there are considerable drug-drug interactions with a multitude of other drug classes that are metabolized by hepatic cytochrome P450 enzymes (Tables 4 and 5). These are too numerous to describe in detail, but transplant clinicians should be mindful of the potentiation of calcineurin inhibitors by azoles. The degree of potentiation differs according to the azole. For example, there is an approximately 20% increase in cyclosporine levels with fluconazole 400 mg but an approximately 50% increase in cyclosporine levels with itraconazole and voriconazole. For voriconazole, the increase in cyclosporine levels is similar to that caused by itraconazole, but the increase in tacrolimus levels is even greater.

The first-generation azoles—clotrimazole, ketoconazole, and fluconazole—have excellent activity against *Candida* species. Multiple controlled trials have shown these agents to be effective as therapy for oropharyngeal candidiasis. Because of a lack of systemic effect, clotrimazole is used only for mucosal infections. Ketoconazole, the first systemic azole, has largely been replaced by fluconazole because of variable bioavailability and dependence on gastric acidity for maximal absorption.

Fluconazole is available as both an oral and intravenous formulation and has excellent bioavailability (>90%) independent of gastric acidity (Table 2). The drug half-life is approximately 30 hours, facilitating once-daily dosing. Fluconazole is metabolized by cytochrome P450 3A4 isoenzymes in the liver and the gastrointestinal tract and is excreted renally. Dose adjustments are necessary when the creatinine clearance decreases to <50 mL/min. Routine dosage adjustments are not required for liver dysfunction; however, if hepatic transaminases increase in the presence of fluconazole, this may represent drug-induced toxicity. The standard dose of fluconazole in the treatment of *Candida* mucosal infections is generally 100 to 200 mg/d. For candidemia caused by susceptible *Candida* species, doses of 400 mg are recommended, with the exception of *C. glabrata* species, for which higher doses of up to 800 mg/d may be necessary. For chil-

Table 4. *Clinically Relevant Drug Interactions with Systemic Antifungal Agents Resulting in an Increase in the Serum Concentration of the Concomitantly Administered Drug*

Type of Drug Interactions	Fluconazole PK	Itraconazole PK	Voriconazole PK	Amphotericin B PD
Concomitant drug whose serum concentration is increased by the antifungal agents	All *trans*-retinoic acid Antihistamines (astemizole, terfenadine) Benzodiazepines (eg, midazolam, triazolam) Calcineurin inhibitors (ie, cyclosporine, tacrolimus) Carbamazepine HMG CoA reductase inhibitors (eg, simvastatin) Hypoglycemic agents (eg, glimepiride) Opioids (eg, alfentanil) Phenytoin Rifamycins (eg, rifampin, rifabutin) Sirolimus Warfarin	Antiarrhythmics (eg, dofetilide, quinidine*) Antihistamines (astemizole, terfenadine*) Antineoplastic agents (eg, vinca alkaloids, busulfan, cyclophosphamide, docetaxel, etoposide) Benzodiazepines (eg, oral midazolam, triazolam)* Calcineurin inhibitors (eg, cyclosporine, tacrolimus) Cisapride* Coicosteroids (oral/inhaled/IV) Digoxin HMG CoA reductase inhibitors (eg, atorvastatin, lovastatin,* simvastatin)* Opioids (eg, fentanyl) Pimozide* Quinidine Sirolimus Warfarin	Antihistamines (astemizole, terfenadine)*† Antineoplastic agents† (metabolized via CYP3A4, 2C9, and 2C19 isoenzymes) Benzodiazepines‡ Calcineurin inhibitors (cyclosporine, tacrolimus) Calcium channel blockers‡ (dihydropyridine class) Ergot alkaloids* HMG CoA reductase inhibitors‡ Hypoglycemic agents‡ NNRTIs‡ Omeprazole Phenytoin Protease inhibitors‡ Rifamycins (rifabutin, rifampin)[1] Sirolimus* Warfarin	Aminoglycosides Antineoplastic agents (eg, cisplatin, bleomycin) Cidofovir Calcineurin inhibitors (cyclosporine, tacrolimus) Flucytosine Foscarnet

PK indicates pharmacokinetics; PD, pharmacodynamics; CYP, cytochrome P450; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IV, intravenous; NNRTI, non-nucleoside reverse transcriptase inhibitors.

*Contraindicated.

†Interaction hypothesized on the basis of metabolism.

‡Data derived from in vitro studies.

dren, dosing for serious *Candida* infections is 6 mg/kg/d.

Fluconazole has a favorable safety profile compared with other antifungal agents and is well tolerated, with few severe toxicities. One of the most common toxicities is an increase in hepatic transaminases. Other side effects include nausea and vomiting (at the higher doses) and rashes. Because of the hepatic metabolism, fluconazole is subject to several drug interactions, where it may increase the serum concentration of concomitantly administered medications; conversely, its own concentration may be altered by the concomitantly administered drug (Tables 4 and 5).

Fluconazole is highly active against *Candida* isolates. There is also activity against *Cryptococcus* species, histoplasmosis, and coccidiomycosis. Randomized trials and case-controlled studies have shown fluconazole to be highly effective as therapy of systemic *Candida* infections (Table 6), with response and survival rates comparable to those of amphotericin B [45,46]. Time to clearance of *Candida* bloodstream infections is similar although slightly slower than with amphotericin

Table 5. *Clinically Relevant Drug Interactions with Antifungal Agents: Effects of Other Drugs on the Pharmacokinetics of Antifungal Agents*

Variable	Fluconazole	Itraconazole	Voriconazole	Amphotericin B
Concomitant drugs that decrease serum concentrations of systemic antifungal agents	Phenytoin Rifabutin Rifampin	Phenytoin Rifampin Rifabutin	Barbiturates (long acting)*† Carbamazepine† NNRTIs‡ Phenytoin Protease inhibitors‡ Rifabutin* Rifampin*	Nil

*Contraindicated per package insert.

†Interaction hypothesized on the basis of metabolism.

‡Data derived from *in vitro* studies.

Table 6. *Randomized Comparative Trials for Therapy of Systemic Candida Infections*

Study	% Success				
	Fluconazole	Amphotericin B	Combination*	ABLC	Caspofungin
Rex [46]	70	79			
Anaissie [28]		68		63	
Phillips [45]	50	56			
Rex [84]	56		68		
Mora-Duarte [62]		56			66

*The combination of amphotericin B with fluconazole.

B. It is important to note that most of these clinical trials were conducted in nonneutropenic patients, and there continues to be a paucity of data in neutropenic patients. Thus, many experts continue to recommend use of amphotericin B as preferable to fluconazole in treatment of the neutropenic patient with systemic candidiasis.

Itraconazole. This azole has activity not only against *Candida* and *Cryptococcus* species, histoplasmosis, blastomycosis, and coccidiomycosis, but also against *Aspergillus* species. Two reviews of treatment practices of aspergillosis have illustrated its utility [47,48]. Unfortunately, there is a paucity of controlled trials of itraconazole in the therapy of invasive *Candida* and *Aspergillus* infections; thus, it is unclear as to how it compares with amphotericin B.

Itraconazole is less reliably and less well absorbed by mouth (55% for the solution and much less for the oral capsule) and has substantial interpatient variability. The intravenous formulation is well tolerated. Itraconazole demonstrates nonlinear pharmacokinetics; thus, small increases in dose can lead to significantly increased serum concentrations. Itraconazole is metabolized primarily by the cytochrome P450 3A4 isoenzyme system and therefore is also subject to a number of drug interactions with other medications (Tables 4 and 5). Itraconazole oral solution can be difficult to tolerate, especially in the presence of mucositis. Itraconazole also has substantial negative inotropic effects. These cardiac effects can be particularly problematic in patients who have received anthracyclines or in those receiving concomitant high-dose cyclophosphamide. Although itraconazole is metabolized by the liver, its intravenous formulation is suspended in cyclodextrin, which is cleared by the kidneys. Accordingly, it should be avoided in patients with renal impairment (creatinine clearance $<$30 mL/min). The oral formulation can be prescribed irrespective of creatinine clearance.

Voriconazole. Voriconazole has the broadest antifungal spectrum of all the licensed azole antifungals [49-53]. In clinical trials, responses have been noted in *Aspergillus*, *Candida* (including fluconazole-resistant *Candida* species), *Fusarium*, and *Scedosporium* infections. A major gap in its coverage is a lack of activity against *Zygomycetes*. A randomized trial comparing amphotericin B and voriconazole as first-line therapy of invasive aspergillosis demonstrated voriconazole to be more effective than amphotericin B, with higher response rates and better overall survival [54]. In addition, voriconazole was associated with fewer toxicities and greater tolerance. Some have criticized this study, believing that a lipid amphotericin B would have been a fairer comparison. One can only speculate whether that would have changed the results, but, as noted previously, the only randomized comparison between a lipid amphotericin and amphotericin deoxycholate did not show an improvement in either response or survival [31].

Voriconazole avoids some of itraconazole's shortcomings. It has excellent bioavailability (96%), without dependence on gastric acidity (Table 2). There are no deleterious inotropic effects, unlike with itraconazole. The intravenous formulation's excipient is a cyclodextrin, as with itraconazole, although it is a different molecule. Accordingly, as with itraconazole, intravenous voriconazole should be avoided in patients with compromised renal function (creatinine clearance $<$50 mL/min). When oral voriconazole is prescribed, no dosage modification is required for renal insufficiency. For individuals aged $\geq$12 years, the pharmacokinetics are nonlinear. For children under the age of 12 years, the kinetics are linear and clearance is more rapid, necessitating higher doses to achieve areas under the curve similar to those of older patients [55]. Voriconazole penetrates the cerebrospinal fluid and has documented activity in cerebral fungal infections, including disseminated *Fusarium* species infections and disseminated *Aspergillus* infections [56].

There are 2 unique toxicities with voriconazole that are not typically seen with other azoles. One is photopsia, a visual disturbance manifested as a visual halo, light aura, or blurring of visual objects. This occurs in 15% to 45% of patients in clinical trials, typically 30 to 60 minutes after the drug is administered (either orally or intravenously), lasts 30 to 60 minutes, is most frequent early after the start of therapy, and usually abates over time. Despite this, patients should be advised not to drive at night while

taking voriconazole, and, similarly, they should avoid potentially hazardous tasks if they perceive any change in vision. A second reaction is photosensitivity, which can occur in up to 5% of patients given the drug in the outpatient setting. Neither reaction is severe, results in permanent dysfunction, or necessitates discontinuation of the drug.

One of the most important considerations with voriconazole is the potential for drug interactions to occur (Tables 4 and 5). Voriconazole is metabolized by 3 cytochrome P450 isoenzymes: CYP3A4, CYP2C9, and CYP2C19. Many of the commonly used drugs in immunocompromised patients have been evaluated in pharmacokinetic drug interaction studies before Food and Drug Administration approval.

One other triazole is in clinical development. Posaconazole, currently available only in an oral formulation, is in phase III clinical trials. Its antifungal spectrum seems to be similar to that of voriconazole, but additionally it is active against the agents of mucormycosis, in contrast to voriconazole. It does not cause the visual changes seen with voriconazole.

Echinocandins

The mechanism of action of the echinocandins is inhibition of β (1,3)-glucan synthase, which leads to interference in the synthesis of glucan, a major constituent of the fungal cell wall and a unique fungal target. Reduced glucan makes the fungal cell vulnerable to osmotic lysis.

Caspofungin. This is the only licensed member of this family of antifungals. Caspofungin has excellent in vitro activity against *Candida* (including azole-resistant species) and *Aspergillus* species [51,52,57-59]. In vitro testing studies have raised concerns of lower activity against several non-*albicans Candida* species, but whether these in vitro findings are clinically important is unclear because the clinical responses for these species seem to be comparable to those with amphotericin B. It was first licensed on the basis of clinical responses noted in patients with invasive aspergillosis who had not responded to amphotericin B or who were intolerant of amphotericin B [60]. Two randomized trials, one in *Candida* esophagitis and the other in systemic candidiasis, have demonstrated excellent clinical activity [61,62] comparable to that of fluconazole and amphotericin B, with substantially less toxicity than amphotericin B. Caspofungin is not active against *Cryptococcus*. There is little information as to the activity of this agent against other fungal pathogens.

Caspofungin is poorly absorbed orally and is available only in an intravenous formulation. The half-life of the drug is 12 to 16 hours, permitting once-daily dosing. Dose adjustments are not needed in renal impairment or in mild hepatic insufficiency (Child-Pugh score of 5 or 6) but should be adjusted in patients with moderate hepatic impairment (Child-Pugh score of 7-9). There are no published data in patients with severe hepatic insufficiency (Child-Pugh score of >9). To date, the emergence of antifungal resistance has not been observed in clinical practice for therapy of *Candida* and *Aspergillus* infections.

Few toxicities have been noted with caspofungin. One notable caveat is an increase in hepatic transaminases seen in healthy volunteers given cyclosporine. This did not result in severe sequelae and was reversible on withdrawal, but this has necessitated a warning that clinicians should use caspofungin in patients receiving concomitant cyclosporine only if the benefit is deemed more important than the risk. Cyclosporine seems to increase caspofungin blood levels up to 30%. Dosing in children is still being worked out, although preliminary results indicate that a dose of at least 1 mg/kg is required, and computer modeling suggests that dosing on the basis of body-surface area achieves more predictable blood concentrations (Walsh TJ, unpublished observations, 2003).

Two other echinocandins are in clinical trials. Micafungin seems to have a similar antifungal spectrum of activity and toxicity profile as caspofungin [63]. No hepatic transaminase increases have been noted in patients receiving micafungin and cyclosporin, unlike with caspofungin. Anidulafungin is in clinical trials.

Biologics. In vitro experiments indicate that antifungal agents in combination with phagocytic host cells have additive effects [64-69]. Attempts to exploit these observations have been made by using exogenous cytokines to boost these effects. Such experiments suggest that agents such as granulocyte-macrophage colony-stimulating factor and γ-interferon may have salutary effects against *Aspergillus* [64,68,70-75]. To date, there are insufficient clinical trial data to determine whether these are clinically important. Accordingly, consensus panels do not recommend their routine use. Another modality potentially useful in infected neutropenic patients is granulocyte transfusions. With granulocyte colony-stimulating factor priming of a donor, large numbers of granulocytes can be obtained, and administration to recipients can have substantial boosts in the circulating neutrophil count. Anecdotal evidence suggests a possible benefit [76-78]. These have been generally well tolerated. Again, lack of controlled trials makes the role of these unclear.

THE STRATEGIES

As important as the drugs we use are for the prospects for success, the manner in which we use them is also extremely important. Three general approaches have been used: treatment, empirical therapy

Table 7. *Randomized Comparative Trials for Therapy of Invasive Aspergillus Infections*

Author	Test Agent	Comparator	Response Rates		Survival Rates	
			Test Agent	Comparator	Test Agent	Comparator
Ellis [37]	L-amph 1 mg/kg	L-amp 4 mg/kg	64%	48%	78%	80%
Bowden [31]	ABCD 6 mg/kg	Amph (1-1.5 mg/kg)	35%	35%	64%	55%
Herbrecht [54]	Vori 6 mg/kg LD, then 4 mg/kg every 12 h	Amph (1-1.5 mg/kg)	52.8%*	31.6%*	70.8%	57.9%

L-amp indicates liposomal amphotericin B; ABCD, amphotericin B colloidal dispersion; Amph, amphotericin B deoxycholate; Vori, voriconazole; LD, loading dose.

*$P < .05$.

for suspected infection, and prophylaxis for those at high risk for IFI. The reason the last 2 have been considered is the generally poor outcomes with treatment regimens. Studies have demonstrated the utility of such approaches in HCT and other situations [79-82]. These comments will focus on approaches to *Candida* and *Aspergillus* infections only.

Treatment

A number of treatment studies have been conducted for *Candida* and *Aspergillus* infection, and these have formed the basis for treatment guidelines by consensus panels [39,83]. These must be interpreted in the light of recent studies that likely alter some of those recommendations formulated 4 years ago.

Candida. Most experts recommend the removal of the central venous catheter or any other foreign body in an infected patient whenever possible [39] because drugs often do not effectively penetrate biofilms on plastic surfaces harboring organisms. There are several excellent options for antifungal therapy supported by randomized trials (Table 6). Amphotericin B has historically been the gold standard. Intolerance and the potential for severe nephrotoxicity makes it an unpalatable choice in patients receiving calcineurin inhibitors [9]. ABLC has been shown to be as effective as amphotericin B, with considerably less toxicity [28]. Accordingly, a lipid formulation would be preferable to amphotericin B deoxycholate. Caspofungin is another excellent choice [62], but caution is necessary if the patient is receiving cyclosporine, as noted previously. One option is to switch cyclosporine to tacrolimus if the clinician wishes to use caspofungin and deems a change in calcineurin inhibitor acceptable. Fluconazole would be inappropriate for a patient who develops candidemia while receiving fluconazole prophylaxis, but it would be an excellent choice for a patient who was not receiving fluconazole and the isolate is susceptible [45,46]. The risk that one may be treating a nonsusceptible *Candida* pathogen is the major factor that one should weigh in deciding whether fluconazole is appropriate for initial therapy. Often, several days pass from notification of a positive culture before the isolate is speciated. Susceptibility testing is not widely available at present, and testing (even if it is available) adds even more time. Knowledge of the species provides a good estimate of susceptibility, with *C. krusei*, *C. glabrata*, and *C. dubliniensis* not reliably susceptible and all others susceptible [39]. Accordingly, one option is to start with a lipid amphotericin B or caspofungin and, once the organism is speciated and the patient stabilizes, change to fluconazole for susceptible isolates to complete therapy. One study has evaluated combination therapy for candidemia in nonneutropenic patients [84] and is discussed below.

The duration of therapy is problematic, and there is no clear guidance from published literature. Generally, one should continue treatment until resolution of signs and symptoms, clearance of cultures, improvement of radiologic manifestations, and improvement of the host defenses that contributed to the infection.

Aspergillus. Early detection and prompt initiation of antifungal therapy are key. Although amphotericin B deoxycholate has been the gold standard, it is poorly tolerated in allogeneic HCT patients, and success rates are poor. The lipid amphotericin B products are preferable in the allogeneic HCT patient (for tolerance reasons), but results are not convincingly better in terms of success or survival [31] (Table 7). There are no controlled first-line treatment trials with itraconazole or caspofungin. Recently, voriconazole followed by other licensed antifungal therapy (for patients with intolerance or lack of response) was found to be superior to amphotericin B deoxycholate in terms of response, survival, and tolerance, both overall and in the HCT subgroup [54]. Accordingly, voriconazole currently offers the best prospect for success and tolerance, on the basis of clinical trial data for first-line therapy. A lipid amphotericin B is an acceptable alternative for those who cannot receive voriconazole because of intolerance; when it is used, it should be given at a dose of 4 to 6 mg/kg/d. For patients whose infection progresses with voriconazole, one of the lipid amphotericin B products, caspofungin, or perhaps itraconazole are good second-line treatment op-

Table 8. *Comparison of Various Antifungal Agents Used as Empirical Therapy during Neutropenic Fever Suspected to Be Caused by Invasive Fungal Infections*

Author*	Test Agent	Control Comparator	Success Rates		Rates of Documented Emergent IFIs	
			Test Agent	Control	Test Agent	Control
Pizzo [86]	Amph	No antifungal	Not stated	Not stated	5.5%	31%
EORTC [85]	Amph	No antifungal	69%	53%	1.5%	9.4%
White [32]	ABCD	Amph	50%	43%	3.1%	3.2%
Walsh [35]	L-amph	Amph	50%	49%	3.2%†	7.8%†
Boogaerts [87]	Itra	Amph	47%	38%	2.6%	2.6%
Walsh [88]	Vori	L-amph	26%	31%	1.9%	5%
Wingard [34]	ABLC	L-amph (3/5)‡	33%	40%/42%	3.8%	3.6%/2.5%
Fleming [33]	ABLC	L-amph	63%§	39%§	Not stated	Not stated
Prentice [25]	L-amph (1/3)‡	Amph	58%/64%§	49%§	2.6%/1.7%	2%

Amph indicates amphotericin B deoxycholate; ABCD, amphotericin B colloidal dispersion; L-amph, liposomal amphotericin; Vori, voriconazole; Itra, itraconazole; EORTC, European Organization for Research and Treatment of Cancer; ABLC, amphotericin B lipid complex.
Trials in which fluconazole was the comparator were excluded, because HCT patients are given fluconazole prophylaxis.
†Proven infection rates only: when combining both probable and proven IFIs, 4.95% versus 8.7%.
‡Two doses of study drug (mg/kg/d), each evaluated in separate arms.
§$P < .05$.

tions. In addition to pharmacologic therapy, resection of localized infracted tissue should be considered (see below) [83].

Empirical Antifungal Therapy

Two small controlled trials of amphotericin B [85,86] demonstrated the capability of empirical antifungal therapy to reduce fungal infections in neutropenic patients with persistent unexplained fever not responsive to antibiotics (Table 8). Initially, amphotericin B was begun after 7 days of antibiotics, but in successive studies, the start of antifungal therapy was pushed earlier to 3 to 4 days of persistent fever, although no study has convincingly documented the need for an earlier start. Because of the considerable toxicity of amphotericin B, as noted previously, alternatives to amphotericin B have been evaluated in controlled trials. In subsequent trials of empirical antifungal therapy in which 2 active treatments were compared, the primary end point most commonly used has been "success": a composite of several parameters, including defervescence, absence of breakthrough IFI, survival, and absence of toxicity requiring discontinuation of the study drug. The problem with interpretation of the results of such trials is that success is influenced by multiple factors, many of which may have nothing to do with IFIs. The rates of documented IFIs generally are too low (because active drug is used in both arms and therapy is started early in neutropenia) to determine relative efficacy from these trials. Typically, more information is gained about relative toxicity profiles than about comparative efficacy from empirical therapy studies.

Lipid formulations of amphotericin B (ABCD and liposomal amphotericin B) have demonstrated similar success rates as amphotericin B, with considerably less toxicity [32,35] (Table 2). Intravenous itraconazole has been compared with amphotericin B for persistent neutropenic fever [87]; there was a trend toward a higher rate of success with itraconazole. Voriconazole was compared with liposomal amphotericin B; success was slightly less frequent with voriconazole, but fewer breakthrough IFIs occurred with voriconazole [88]. Recently, caspofungin has also been shown to provide a suitable alternative, with less toxicity than liposomal amphotericin B [89].

Prophylaxis

Amphotericin(s). Leukemic patients with prior *Aspergillus* infections almost uniformly experience reactivated infection on further antileukemic therapy or subsequent HCT once they are in remission. In the past, the extremely high case fatality rate led many centers to exclude such patients from consideration for HCT. The use of treatment doses of amphotericin B (1 mg/kg/d) as secondary prophylaxis allowed successful further antileukemic treatment and HCT without exacerbation [90]. Primary prophylaxis (for patients without prior IFI) with lipid amphotericin products has been studied in only a limited manner; trials that have evaluated liposomal amphotericin B have been too small to adequately test this strategy [91].

Low doses of amphotericin B have also been tested for primary prophylaxis [92,93], but this approach provides protection only against *Candida* and not against *Aspergillus*. Because fluconazole is more tolerable for this purpose, this approach is not widely used.

Fluconazole. Prospective randomized trials have demonstrated the effectiveness of fluconazole prophylaxis in HCT recipients when it is given from the start of the conditioning regimen until engraftment

[94,95]; it reduces invasive *Candida* infection rates from 16% to 3%. In one trial, prolonged administration (until day 75) not only had a similar antifungal benefit, but also was associated with a survival advantage [96] that persisted even beyond the cessation of fluconazole [97]. Initial studies evaluated a dose of 400 mg/d, but one study demonstrated that 200 mg/d was also effective [98]. Emergence of resistance has not been reported. However, isolated reports of outbreaks of fluconazole-resistant organisms, such as *C. krusei* and *C. glabrata*, have been reported in several HCT centers [42-44]. Fluconazole prophylaxis has been endorsed by consensus guidelines developed by the Centers for Disease Control, the American Society of Blood and Marrow Transplantation, and the Infectious Disease Society of America [99].

Itraconazole. Two studies have evaluated itraconazole as long-term antifungal prophylaxis after allogeneic BMT, with the goal of reducing not only *Candida* but also *Aspergillus* infections [100,101]. In 1 of the 2 itraconazole trials [100], a reduction in IFIs was noted (in comparison to fluconazole), but there were inadequate numbers of *Aspergillus* cases to determine how effective it was as a prophylaxis against aspergillosis. Of concern, there was an excess of deaths (29.5% versus 18%) and of adverse events (6 versus 1), and this led to discontinuation of the drug in the itraconazole group. The second randomized trial comparing itraconazole and fluconazole [101] used a higher dose of itraconazole to ensure therapeutic blood concentrations. The trial was stopped prematurely after nearly 300 patients were enrolled (substantially more enrollees than in the earlier study) because of excessive toxicity in the itraconazole arm. A significant excess of renal and hepatic toxicity was noted in the patients in the itraconazole arm. Thirty-six percent of patients taking itraconazole stopped the drug because of toxicity. There was no reduction in the frequency of fungal infections in the itraconazole arm, although in a post hoc subset analysis of those who were able to tolerate it, there were fewer infections in the itraconazole arm. Taking both itraconazole HCT trials together, there currently are insufficient data to state definitively that itraconazole is safe and effective in the allogeneic BMT setting for long-term prophylaxis.

Micafungin. This as-yet unlicensed echinocandin was tested in a randomized comparison with fluconazole as prophylaxis in HCT patients during the preengraftment period [102]. It was found to offer protection similar to that of fluconazole against IFIs and fewer persistent unexplained febrile episodes requiring empirical trials of amphotericin B. It was well tolerated. There were too few *Aspergillus* infections to ascertain its protective capability against that pathogen.

Infection-Control Measures

Candida organisms are generally endogenous, commensal colonizers of skin and mucosal surfaces; most infections arise from the patient's own flora. However, some studies have suggested patient-to-patient transmission in hospital environments (presumably by health-care workers) in units housing transplant recipients, leukemia patients, intensive care unit patients, and surgical patients. Thus, hand washing is an important facet of infection control. Outbreaks of infections in which nosocomial transmission is a possibility should be investigated by the hospital infection-control team. Molecular testing for DNA polymorphisms can be quite useful to determine whether 1 or more strains are present in multiple patients [103]. Point sources of infection that have been identified in investigations of outbreaks include intravenous solutions, medications, and plastic tubing.

Because *Aspergillus conidia* are exogenous organisms present in the environment and are primarily airborne, transmission of organisms can occur in the hospital environment during construction, renovation, or other activities in which organisms can be spread and inhaled by susceptible immunocompromised patients. Accordingly, high-efficiency air filtration is important in hospital rooms in which highly susceptible patients reside, to prevent outbreaks. These include HCT recipients, and the routine use of high-efficiency particulate air filters is recommended in consensus guidelines [99]. The use of high-efficiency masks worn during transport when patients leave their rooms may also be helpful [104]. Recently, patient shower facilities have been implicated as potential sources of nosocomial *Aspergillus* acquisition [105,106]. Avoidance or cleaning procedures have been proposed to reduce the risk to susceptible patients [107].

Adjunctive Therapies

Surgical excision should be considered in patients with pulmonary *Aspergillus* infections in which cavitary or necrotic tissues are persistent or in which lesions are centrally located and catastrophic hemorrhage may occur because of invasion of the pulmonary vasculature [83]. Catheter removal should be promptly undertaken for all candidemic patients if possible [39,108]. Several studies suggest more rapid clearance of organisms from the bloodstream with catheter removal, although a recent survey noted the limitations of the data on which this recommendation is based and suggested that clinical judgment be used to identify those in whom the catheter should be retained, taking into consideration the risks of its removal and its potential benefits [109]. *C. parapsilosis* is frequently associated with vascular catheters, and

catheter removal is especially important for this pathogen.

NEW DIRECTIONS

New Diagnostics

Treatment outcomes have been severely compromised by the inability to make the diagnosis early and start therapy promptly. The evidence for this is largely indirect. Outcomes for *Candida* infections are poorer when tissue sites are involved as well as the bloodstream, and poorer outcomes are evident in patients with greater physiological compromise, as measured by acute physiology and chronic health evaluation scores. For *Aspergillus* infections, involvement of more than 1 organ site is associated with poorer outcomes than involvement of only 1 site. Moreover, outcomes of *Aspergillus* infections identified early, as evident by the presence of early radiologic manifestations (the halo sign on chest computed tomographic scan), are better [110].

For these reasons, the development of new rapid diagnostics has been a high priority for improving treatment outcomes. Serologic assays are clinically available for cryptococcosis, histoplasmosis, and coccidiomycosis, but until recently no similar assays have been available for *Candida* and *Aspergillus* infections.

The galactomannan assay has been recently approved for use in the diagnosis of *Aspergillus* infections. This double sandwich enzyme-linked immunosorbent assay detects the concentration of the soluble galactomannan antigen, which is present in the circulation of patients with invasive *Aspergillus* infections, and compares the optical density index with that of a known positive serum; a calculated index determines positivity. Receiver operator curves suggest that an optical density index of 0.5 is optimal for most circumstances [111]. The test has been available in Europe for years, but it has only recently been approved for use in the United States. It is important to note that the threshold of positivity of the licensed US test is set lower than that of the European licensed test. True positivity necessitates repeating the assay, and the index must exceed 0.5 on 2 determinations. Initially, 2 separate specimens were required for this, but data indicate that most of the false positives can be resolved by merely repeating the test on the same specimen. If the test is negative, no repeat is required. In data presented to the Food and Drug Administration, sensitivity in the diagnosis of invasive *Aspergillus* infections was approximately 80%, and specificity was also approximately 80%. In a prospective evaluation of the assay in Europe in allogeneic HCT patients in whom the test was performed twice weekly, the sensitivity and specificity were approximately 90% [112]. Most importantly, in nearly two thirds of infections, the assay was positive before currently available diagnostic methods with clinical, radiologic, and other diagnostic tools. The test is recommended to be performed twice weekly during the period of vulnerability. Certainly, this is a welcome addition to the tools available to the clinician.

Positivity of the galactomannan assay also occurs with infections caused by *Penicillium* and *Alternaria* (true positives), but the rarity of those pathogens means that it is fairly specific for aspergillosis. Concerns have been raised questioning the utility of the galactomannan assay in specific circumstances. Patients receiving antimold agents empirically or prophylactically may develop invasive *Aspergillus* infection with galactomannan levels in the blood below the threshold of positivity in the licensed test [111]. Development of antibody by some patients may limit its usefulness in such patients [113]. The test has not yet been adequately studied in children.

The galactomannan assay has also been evaluated for detection of *Aspergillus* antigens in other specimens, including cerebrospinal fluid and bronchoalveolar fluid specimens. The early results are promising, but more data are needed to clarify whether these will be useful in clinical practice. Further, although reporting of the commercial test results will be qualitative (positive or negative), quantitative results may prove to be more informative, with the degree of change giving additional information that may improve the performance of the assay. Moreover, testing of serum specimens obtained after the start of antifungal therapy may also provide early information as to the likelihood of response or whether alternative treatment should be considered. These questions require more investigation.

Another assay, the β-glucan assay, is in clinical testing [114] (Ostrosky-Zeichner L, unpublished data, 2003). This antigen is broadly expressed in multiple fungal genera. Thus, it can provide complementary information to the galactomannan assay, which is limited to just *Aspergillus* among pathogens common to humans in the United States and Europe. Its sensitivity and specificity are currently being evaluated in prospective testing.

Several polymerase chain reaction assays that detect fungal gene products present in a broad spectrum of fungal pathogens have been tested [115]. In 1 case, a polymerase chain reaction assay was used to guide empirical antifungal therapy, and this resulted in a sparing of febrile patients not infected by fungi from receiving antifungal agents [115]. Additional trials with this technology are required.

Combination Therapy

Early studies found that combination therapy with amphotericin B plus flucytosine was more effective

than monotherapy with amphotericin B for cryptococcal meningitis. Unfortunately, despite some in vitro data suggesting that combining amphotericin B with rifampicin or flucytosine may be more effective for *Aspergillus* or *Candida*, there have been no confirmatory clinical results.

In vitro susceptibility assays demonstrate additive or synergistic activity of polyenes plus echinocandins against *Aspergillus* [116,117]. Similarly, in vitro assays suggest additive effects of voriconazole plus an echinocandin [118]. Animal models that have tested combinations suggest that the in vitro observations may translate into an in vivo benefit [119-123]. Notwithstanding, the animal models have had to test the drugs in subtherapeutic doses. Whether similar additive effects are also present when both drugs are used at full doses is not known. There is a paucity of clinical data in which combination therapy has been compared with monotherapy [124,125]. Accordingly, at present, it is not possible to discern whether combination therapy is better than monotherapy, and it is generally not recommended [126]. It is important to recognize that not all combinations are additive, and some may in fact be antagonistic [127-131]. Accordingly, cautious evaluation of this promising topic is necessary.

There is 1 notable exception to these cautions. Rex et al. [84] evaluated combination therapy for the treatment of candidemia in nonneutropenic adults, comparing amphotericin B plus fluconazole versus high doses (800 mg/d) of fluconazole alone. There was a nonsignificant trend to higher success rates with the combination therapy compared with monotherapy (69% versus 56%; $P = .08$) and better clearance of the bloodstream infection by the combination therapy ($P = .02$). Unfortunately, there was also more toxicity with the combination therapy, and this mitigated the net benefit.

CONCLUSIONS

IFIs are a leading cause of morbidity and mortality after HCT. New drugs (voriconazole and caspofungin) and new formulations of older drugs (the lipid formulations of amphotericin B and intravenous itraconazole) have expanded our therapeutic options, permitting safer formulations (lipid formulations of amphotericin B), more reliable drug delivery (intravenous formulation of itraconazole), broader spectra of activity (oral and intravenous formulations of voriconazole), and novel mechanisms of action (caspofungin). Knowledge of specific spectra of activity of the various agents and recognition of toxicities attendant to the specific agents are both necessary in choosing which agent is optimal for specific pathogens in different patient circumstances. A high state of vigilance is necessary for early detection—a job perhaps made easier by new diagnostics (the galactomannan assay). It is hoped that these new drugs and new detection methods will usher in a new generation of antifungal therapy that will result in improved outcomes.

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